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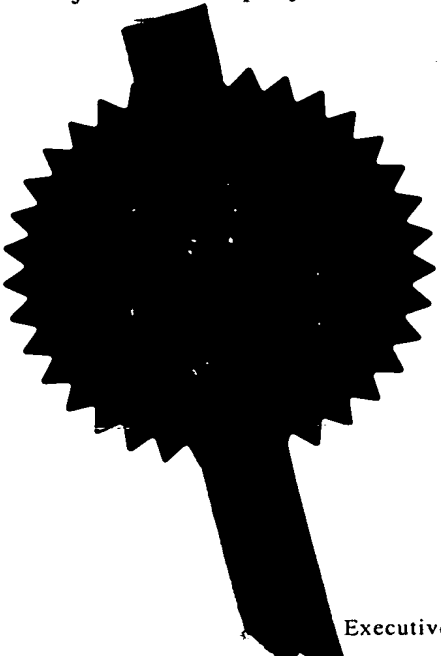
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Signed *Andrew Gensy*
Dated 23 November 1999



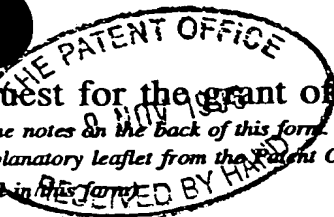
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Request for the grant of a patent
(See the notes on the back of this form. You can also get
an explanatory leaflet from the Patent Office to help
you fill in this form.)



1. Your reference

REP05849GB

2. Patent application number

(The Patent Office will fill in this part)

9824570.7

3. Full name, address and postcode of the or of
each applicant (underline all surnames)

Microscience Limited
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number (if you know it)

If the applicant is a corporate body, give the
country/state of its incorporation

United Kingdom

073044546001

4. Title of the invention

VIRULENCE GENE AND PROTEIN,
AND THEIR USE

5. Name of your agent (if you have one)

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom
to which all correspondence should be sent
(including the postcode)

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number (if you know it)

745002

6. If you are declaring priority from one or more
earlier patent applications, give the country
and the date of filing of the or of each of these
earlier applications and (if you know it) the or
each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise
derived from an earlier UK application,
give the number and the filing date of
the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right
to grant of a patent required in support of
this request? (Answer 'Yes' if:

YES

- a) any applicant named in part 3 is not an inventor
 - b) there is an inventor who is not named as an
applicant, or
 - c) any named applicant is a corporate body.
- See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description 4

Claim(s) 1

Abstract

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

Any other documents
(please specify)

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date

9 November 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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VIRULENCE GENE AND PROTEIN, AND THEIR USE

Field of the Invention

This invention relates to a virulence gene and protein, and their use. More particularly, it relates to
5 their use in therapy and in screening for drugs.

Background of the Invention

E. coli is an organism that is implicated in septicaemia, meningitis, urinary tract infection, wound infection, abscess formation, peritonitis and cholangitis.
10 It would be desirable to provide means for treating or preventing conditions caused by *E. coli*, e.g. by immunisation.

The *mdoG* gene of *E. coli* K12 is known; see EMBL and Genbank accession numbers X64197 and P33136. *mdoG* encodes a
15 56 kD periplasmic protein of uncertain function, although *mdoG* is required for biosynthesis of membrane-derived oligosaccharides (MDOs). *mdoG* is transcribed as part of an operon with *mdoH*, which functions as a membrane-bound glucosyltransferase during MDO synthesis.

Summary of the Invention

The present invention is based on the discovery of a virulence gene in *E. coli* K1, that has homology with the *mdoG* gene of *E. coli* K12. Accordingly, the present invention provides:

25 the therapeutic use of a peptide encoded by the operon including the *mdoG* gene in *E. coli* K1 or K12, or a homologue thereof in a Gram-negative bacterium, or a functional fragment thereof, e.g. a peptide comprising all or part of the 49-member amino acid sequence defined below;

30 a host transformed to express the peptide or modified to disrupt expression of the gene;

a vaccine comprising such a peptide or the means for its expression, or an attenuated vaccine in which the virulence gene is disrupted;

35 the use of the peptide or corresponding polynucleotide as a target for screening potentially useful

drugs, especially anti-microbials, or as a diagnostic agent in the detection of virulence, e.g. for testing for the presence of virulent coliforms in livestock.

Description of the Invention

5 The virulence gene in *E. coli* K1 was identified by using signature-tagged mutagenesis (STM) to screen an *E. coli* K1 mini-Tn5 mutant bank for attenuated mutants, in a mouse model of systemic infection. Bacteria containing a mini-Tn5 insertion within the virulence gene failed to be
10 recovered from mice inoculated with a mixed population of mutants, and are therefore likely to be attenuated.

 The cloned *E. coli* K1 nucleotide sequence immediately following the mini-Tn5 insertion is as follows:

Length: 147nt

15

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1      GGCAGCGTTG AGCTGGTGGG AATTCCAACC AACGATGAAA CCAACGATAA
51     CATCGTCGCT TACTGGACGC CGGATCAGCT GCCGGAGCCG GGTAAAGAGA
101    TGAAC TTAA ATACACCATC ACCTTCAGCC GTGATGAAGA CAAACTG
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20 A translation of this sequence is as follows:
Length: 49 amino acids

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1      GSVELVEIPT NDETNDNIVA YWTPDQLPEP GKEMNFKYTI TFSRDEDKL
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25 These sequences both show 100% identity to the *mdoG* gene of *E. coli* K12, at nucleotides 1458-1604 and amino acids 346-394 of the latter.

 This demonstrates that the disrupted gene is at least partially identical to the *mdoG* gene of *E. coli* K12.

30 The 49 amino acid sequence also shows homology to the *yhr4* gene of *Pseudomonas syringae*, Swissprot database accession number P20400. The *yhr4* gene is located in the *hrpM* locus and is known to function in phytopathogenicity.

 GCG bestfit analysis at the amino acid level is as
35 follows:

1 GSVELVEIPTNDETNDNIVAYWTPDQLPEPGKEMNFKYTITFSRDEDKL 49
 |.||||||| ||||| | : | |||.|| | | : .. |. :
 94 GTVELVEIPTADETNDNIVAYWKPETLAEPGQEMAFDYRLHWTMQENSI 142

5 The novel gene has been tested for attenuation of virulence, using mixed infections, in a murine model of systemic infection (Achtman et al., 1983, Infection and Immunity, vol 39, pages 315-335), and shown to be attenuated with a complete index (CI) of 0.38 (mean CI
 10 from four mice).

As the *E. coli* K12 *mdoG* gene is transcribed as part of an operon with the *mdoH* gene, it is possible that this attenuation is due to a polar effect on a presumed *E. coli* K1 *mdoH* gene.

15 The *E. coli* K1 *mdoG* gene is likely to be useful both in generating attenuated vaccine strains and as a target for antimicrobials. Given the similarity of the *E. coli* K1 *mdoG* gene to the *hrpM* operon of *P. syringae* (a plant pathogen), the skilled person will appreciate that the
 20 same may be true for *mdoG* homologues in other Gram-negative *Enterobacteriaceae* and in Gram-negative bacteria in general.

For the purposes of this invention, the appropriate degree of homology is typically at least 50%, preferably
 25 at least 60% or 70%, and more preferably at least 80% or 90% (at the amino acid or nucleotide level).

It is evident that *E. coli* K1 strains containing disruptions of the invention are attenuated. The products of the invention may also be immunogenic. They are
 30 therefore useful in therapy, and more particularly as a prophylactic, in a vaccine.

The protein may be purified. It may be sequenced. The corresponding full-length gene can thus be identified. It can thus be prepared by recombinant
 35 technology, by expression in a suitable host. Active fragments and homologues can be identified. Vaccine compositions, including attenuated vaccines, can be

formulated, with carriers and adjuvants as necessary or desired, and used in therapy, to provide an effective immunisation against *E. coli*. In some cases, antibody may be used, for passive immunisation. All these
5 procedures are known to those of ordinary skill in the art, and do not affect the nature of the invention that has been made.

CLAIMS

1. A peptide encoded by the operon including the *mdoG* gene *E. coli* K1 or K12, or a homologue thereof in a gram-negative bacterium, or a functional fragment thereof, for therapeutic use.
2. A peptide according to claim 1, comprising the 49-member amino acid sequence defined herein.
3. A polynucleotide encoding a peptide according to claim 1 or claim 2, for therapeutic use.
4. A host transformed to express a peptide according to claim 1 or claim 2.
5. A vaccine comprising a peptide according to claim 1 or claim 2, or the means for its expression.
6. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene encodes a peptide according to claim 1 or claim 2.
7. Use of a product according to any of claims 1 to 4, for screening potential drugs or for the detection of virulence.
8. Use of a product according to any of claims 1 to 4, for the manufacture of a medicament for use in the treatment or prevention of a condition associated with infection by *E. coli*.

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